pVITRO : Innovative multigenic plasmids for high levels of expression

Use of pVITRO plasmids

- Create gene combinations in a single plasmid
- Improve co-transfection studies
- Co-express two genes stably in mammalian cells

pVITRO is a new family of mammalian vectors with improved features. They allow the co-expression of two or more genes from two different transcription units. pVITRO plasmids can be stably transfected in mammalian cells and are expressed at high levels. Each pVITRO is available with either two multiple cloning sites for cloning of cDNAs, or two reporter genes as control vectors or for the cloning of open reading frames.

Features and Benefits

Common features of pVITRO plasmids:
- Two strong promoters
- IRES from the Foot and Mouth Disease Virus (FMDV) [1]
- Improved hygromycin selection
- Two multiple cloning sites or two reporter genes

Gene combinations in a single plasmid
Coexpression of two or more genes from a single vector is more efficient and convenient than using a monogenic approach. pVITRO are multigenic plasmids that contain two separate transcription units (TU). Each TU can drive the expresion of one or two genes of interest, if using fusion genes.

High-level and constitutive expression of two transgenes:
Each pVITRO plasmid features two strong promoters that drive high levels of expression from two separate TU. Transcriptional interference is minimized by using promoters of different origins or promoters that are coordinately activated (i.e. ferritin promoters), and strong polyadenylation signals.

Similar levels of expression of the inserted genes
The two promoters display comparable strengths, allowing to truly co-express two genes, and avoid the discrepancy of expression that may occur when separate plasmids are used.

Rapid selection of stable transfectants
pVITRO plasmids are selectable with the antibiotic hygromycin B. Selection of stable mammalian clones is usually achieved in less than two weeks.

Single selection marker for E. coli and mammalian cells
The *hph* gene confers resistance to hygromycin B in both

pVITRO1

Mouse and rat EF1α promoters
pVITRO1 plasmids carry two elongation factor 1 alpha (EF1α) promoters, from rat and mouse origins. Similarly to their human counterpart [2], both promoters display a strong activity that yield similar levels of expression.

pVITRO2

Human FerH and FerL composite promoters
pVITRO2 plasmids contain the engineered human ferritin promoters. Ferritin is composed of two chains, light (FerL) and heavy (FerH). The protein, which is expressed in a wide range of cell types, is subject to iron regulation [3]. To eliminate this regulation, the 5'untranslated region (5'UTR) of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee EF1α genes. The activity of both promoters is further increased by the addition of the SV40 and CMV enhancers, yielding activity similar to the CMV promoter.

pVITRO3

CMV-intron A promoter and EF1α/HTLV
pVITRO3 plasmids feature a CMV promoter/enhancer and a EF1α/HTLV composite promoter. The CMV promoter contains the largest intron of CMV (intron A) [4] and is one of the strongest promoters described. The EF1α/HTLV promoter combines the strong EF1α promoter [2] with the 5'UTR from HTLV [5]. This composite promoter is significantly stronger than the native EF1α promoter.

E. coli and mammalian cells. In bacteria, *hph* is expressed from the E. coli EM7 promoter. In mammalian cells, *hph* is transcribed from "Promoter 1", the first promoter after the Ori (see table hereunder), as a polycistronic mRNA and translated via the IRES.

Custom-made pVITRO plasmids

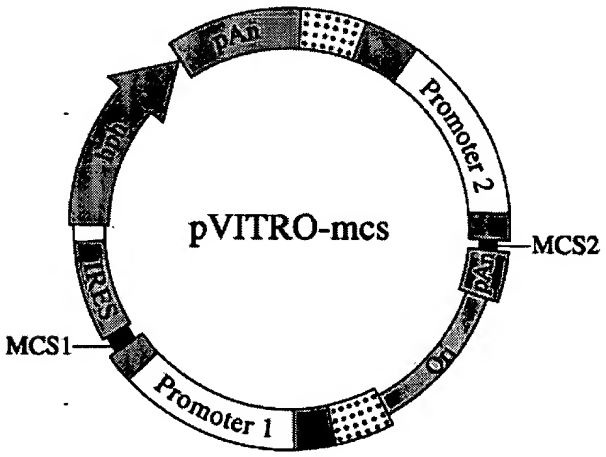
We clone for you:

Any combinations of two genes from our extensive list or with your genes of interest can be created in pVITRO. Select your pVITRO plasmid and the genes in position 1 (upstream of the IRES) and position 2 (upstream of the SV40 polyA) and we will do the cloning for you.

REFERENCES:
1. Ramesh N et al. 1996. High-titer bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. Nucleic Acids Res. 24(14):2697-700
2. Kim DW et al. 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. Gene 91(2):217-23
3. Caughman SW et al. 1988. The iron-responsive element is the single element responsible for iron-dependent translational regulation of ferritin biosynthesis. Evidence for function as the binding site for a translational repressor. J Biol Chem 263(35):19048-52
4. Chapman BS et al. 1991. Effect of intron A from human cytomegalovirus (Towne) immediate-early gene on heterologous expression in mammalian cells, Nucleic Acids Res. 14: 3979-3986
5. Takebe Y et al. 1988. SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol. Cell Biol. 1: 466-472

pVITRO-mcs plasmids

Convenient cloning of cDNAs
pVITRO-mcs plasmids contain two multiple cloning sites (MCS) for the convenient cloning of two cDNAs. Many restriction sites are compatible for ligation, allowing the insertion of a gene of interest into either MCS1 or MCS2.



pVITRO-reporter plasmids

Control vectors, also allowing convenient cloning of ORFs
pVITRO-reporter plasmids express two reporter genes (lacZ and either GFP or PLAP) and can be used as control vectors. Furthermore, all reporter genes are flanked by Nhe I or Avr II at the 3' end and by Nco I or Bsp HI at the 5' end (two compatible restriction sites that encompass the Start codon), therefore allowing the convenient cloning of open reading frames that can be chosen from InvivoGen's pORF list.

pVITRO backbones

	pVITRO 1	pVITRO 2	pVITRO 3
Promoter Region			
Promoter 1	Rat EF1α promoter paired with CMV enhancer	Human FerH / murine EF1α composite promoter paired with SV40 enhancer	CMV-intron A promoter
Promoter 2	Mouse EF1α promoter paired with SV40 enhancer	Human FerL / chimpanzee EF1α composite promoter paired with CMV enhancer	EF1α / HTLV composite promoter
Multiple Cloning Sites			
MCS 1	5' - BspE1 , Bst1107I , BamHI , Bsi WI , AvrII	5' - AgeI , EcoRV , BamHI , MluI , ClaI , AvrII	5' - SmaI , NcoI , BamHI , BstBI , Bsi WI , AvrII
MCS2	5' - AgeI , EcoRV , Bgl II , BsrGI , NheI	5' - SgrAI , FspI , Bgl II , EcoRI , BstBI , XhoI , NheI	5' - SgrAI , StuI , BspHI , Bgl II , ClaI , BsrGI , NheI
Reporter Genes			
Reporter 1	PLAP: Placental alkaline phosphatase	GFP: Green fluorescent protein	GFP: Green fluorescent protein
Reporter 2	LacZ : Beta-galactosidase	LacZ : Beta-galactosidase	LacZ : Beta-galactosidase

Contents and Storage

Each pVITRO plasmid is provided as 20µg lyophilized DNA.

Larger quantities are available. Inquire for more information.

Plasmid is shipped at room temperature and should be stored at –20°C. The plasmid is stable up to one year when properly stored.

Each pVITRO is provided with 4 pouches of E. coli FastMedia™ Hygro (2 pouches for liquid and 2 pouches for solid media).

Product	Quantity	Catalog #
pVITRO1-mcs	20µg	pvitro1-mcs
pVITRO1-PLAP/LacZ	20µg	pvitro1-plaplacz
pVITRO2-mcs	20µg	pvitro2-mcs
pVITRO2-GFP/LacZ	20µg	pvitro2-gfplacz
pVITRO3-mcs	20µg	pvitro3-mcs
pVITRO3-GFP/LacZ	20µg	pvitro3-gfplacz